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ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY

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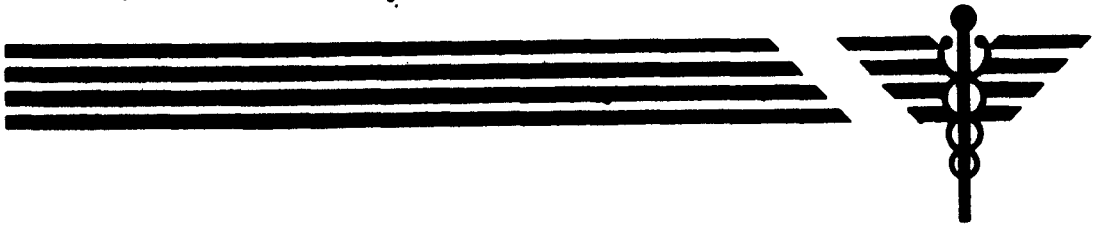
STUDIES ON THE MECHANISM OF BLOOD CLOTTING*

*Subtask under Environmental Physiology, AMRL Project 6-64-12-028 Subtask, Biochemical Aspects of Stress.



RESEARCH AND DEVELOPMENT DIVISION
OFFICE OF THE SURGEON GENERAL
DEPARTMENT OF THE ARMY

REPORT NO. 131

STUDIES ON THE MECHANISM OF BLOOD CLOTTING*

by

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FORT KNOX, KENTUCKY
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ABSTRACT

STUDIES ON THE MECHANISM OF BLOOD CLOTTING

OBJECT

To characterize further the possible identities and functions of the various postulated factors involved in the formation of fibrin.

RESULTS AND CONCLUSIONS

On the basis of the studies reported here, the following scheme of "physiological" fibrin formation is suggested:

LABILE FACTOR
↓

1.	Proconvertin	Platelet Factor, Ca++ →	Convertin
2.	Prothrombin	Convertin, Ca++ →	Thrombin
3.	Fibrinogen	Thrombin →	Fibrin

Factors interfering with the clotting process, such as the anti-platelet factor, anticonvertin, antithrombin and perhaps others, are omitted from the above diagram for reason of simplicity. The labile factor seems to exert an accelerating effect on the rate of conversion of proconvertin to convertin by the platelet factor, but does not appear indispensable for this reaction. Calcium ions seem to play a role in the formation of convertin as well as of thrombin.

On the basis of the findings obtained and of a reevaluation of the various factors postulated to be involved in the formation of thrombin, a list of synonyms for the different factors has been proposed.

RECOMMENDATIONS

In order to have a reliable basis for a more complete understanding of the blood clotting mechanism, it is imperative to isolate the

various factors in chemically pure form. Only when the physiological-chemical reactions brought about by the interaction of these factors can be studied in clearly defined systems, will it be possible to precisely formulate the clotting mechanism.

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STUDIES ON THE MECHANISM OF BLOOD CLOTTING

I. INTRODUCTION

The view has been expressed by Jensen and his collaborators (1) that one phase of the biochemical homeostasis of blood may be related to an equilibrium existing between the rate of fibrin formation and the rate of fibrin dissolution. Fibrin formation and fibrin lysis are then considered as normal physiological processes. Fibrin formation has been correlated to vascular integrity (2).

Since the normal process of fibrin formation may, therefore, be of special physiological importance, its mechanism was explored in greater detail. Physiological fibrin formation is dependent upon the formation of a factor within the blood which has the property of converting prothrombin to thrombin.

There is unanimity of opinion with regard to the role of prothrombin and of its conversion product, thrombin, in the process of fibrin formation. However, there appears to be a great divergency of opinion with regard to the number and function of the factors involved in the formation of thrombin and the mechanism of thrombin formation (3, 4, 5).

Experiments recorded in this report were designed to further elucidate the possible identities and functions of the various postulated factors involved in the formation of thrombin from prothrombin.

II. EXPERIMENTAL

A. Collection of Blood and Plasma

Rabbit blood was collected by cardiac puncture using the multiple syringe technique. The blood was drawn into an ice-cooled, silicone-coated syringe containing 1/10 volume of 0.1 M $K_2C_2O_4$ solution. Blood and oxalate were well mixed, delivered into ice-cooled silicone-coated conical centrifuge tubes and centrifuged at 3000 rpm for 30 minutes at 0° to 5°C. Plasma was drawn off with a pipette, care being taken to minimize contamination of the plasma with platelets. Human blood was collected by venipuncture and processed in the same manner.

B. Preparation of Platelets from Rabbit Blood

Blood was drawn by cardiac puncture as described above using 1/10 volume of 1% di-sodium versenate, pH 7, as anticoagulant. Blood

was delivered into ice-cooled, silicone-coated conical centrifuge tubes and centrifuged at 550 rpm for 25 minutes at 0° to 5°C to remove the erythrocytes. The platelet-rich supernatant plasma was drawn off with an ice-cooled, silicone-coated syringe. This plasma was centrifuged in ice-cooled, silicone-coated conical centrifuge tubes at 1700 rpm for 30 minutes at 0° to 5°C to pack the platelets. The supernatant plasma was decanted and the packed platelets were washed two times with volumes of 0.9% NaCl equal to the original plasma volume. After the second wash, the packed platelets were frozen. Before use, the platelets were thawed, suspended in 0.9% NaCl at the desired concentration and homogenized in a glass homogenizer. Concentration of the platelet homogenate is expressed in multiples of blood concentration.

C. Heated Thromboplastin

Commercial thromboplastin* was made up according to the manufacturer's directions. This suspension was heated at 70°C for 5 minutes, cooled quickly in an ice bath, and then centrifuged to remove the precipitate. The supernatant solution was used in the various tests.

D. Preparation of $BaSO_4$-Eluate

Plasma was stirred for 10 minutes with $BaSO_4$ (Baker, C.P.), 100 mg/ml plasma, centrifuged and the supernatant plasma decanted. The $BaSO_4$ was washed successively with 0.9% NaCl, 0.1 M $Na_2C_2O_4$, and 0.9% NaCl, volumes being equal to the original plasma volume. The $BaSO_4$ was then eluted with 0.1 M sodium citrate solution (1/10 of the original plasma volume), stirred for 10 minutes, centrifuged and decanted. The eluate was dialyzed against 0.9% NaCl solution at 4°C to remove the citrate. The eluate was then diluted with 0.9% saline to the desired concentration.

E. Preparation of Aged Plasma

1. Plasma was obtained as previously described and delivered into 6 inch glass test tubes. These tubes were immersed in a 37°C constant temperature water bath for 24 hours (6).

2. The plasma was divided into 2 ml aliquots in 15 x 125 mm test tubes and allowed to stand unstoppered approximately 10 days at 4°C or until the prothrombin time was delayed to 35 seconds.

*Simplastin, Chilcott Laboratories, Inc., Morris Plains, N.J.

F. Preparation of Various Sera

1. For studies on aged plasma:

a. Fresh blood clotted. Blood was collected in an ice-cooled, silicone-coated syringe with no anticoagulant. The blood was delivered into 50 ml glass centrifuge tubes and incubated 1 hour at 37°C. Clots were rimmed and the serum was separated by centrifugation.

b. Defibrination of $BaSO_4$-treated plasma. $BaSO_4$-treated oxalated plasma was treated with Parke-Davis Topical Thrombin (20 units /ml plasma). The mixture was stirred quickly and incubated for 1 hour at 37°C. The clot was then expressed, and the defibrinated plasma recovered was treated with $BaSO_4$ (50 mg/ml).

2. For studies on convertin: (Rabbit Serum)

a. Fresh serum. Blood was collected in an ice-cooled, silicone-coated syringe with no anticoagulant and delivered into 50 ml glass centrifuge tubes. The blood was allowed to stand at room temperature until 30 minutes after puncture. Serum was separated by centrifugation for 10 minutes at 3500 rpm and was tested immediately.

b. Serum (4-1/2 hrs). Blood was collected as above and allowed to stand with the clot until 45 minutes after puncture. The clot was rimmed and the serum was separated by centrifugation for 30 minutes at 2500 rpm and allowed to stand at room temperature for an additional 3 hours and 15 minutes.

G. Preparation of Labile Factor

The labile factor was prepared according to Owren's method (7) which was modified as follows: The bovine plasma was treated with $BaSO_4$ (1 g/10 ml), defibrinated with thrombin, and treated again with $BaSO_4$ (1 g/20 ml) before the labile factor was isolated. The labile factor preparation thus obtained was found to be free of prothrombin, proconvertin and platelet factor.

H. Preparation of Fibrinogen

Fibrinogen was prepared according to the method of Seegers and associates (8). In the final stages of preparation, the fibrinogen solution was treated with $BaSO_4$ (100 mg per ml) to remove any prothrombin, platelet factor and proconvertin which may have been present.

The fibrinogen was diluted with 0.9% saline so that its concentration in the final test mixture was 0.4%.

The essential details of representative individual experiments are included in the various tables.

III. RESULTS AND INTERPRETATIONS

The data presented in the various tables constitute average values of several identical experiments.

The data presented in Table 1 show that the $BaSO_4$-eluate from oxalated plasma is free of thrombin and that the clotting times of the eluate with either platelets and $CaCl_2$ or with heated thromboplastin are very similar in both one-stage and two-stage tests. These results indicate the presence of a thromboplastin-like factor in the platelets.

Heating the eluate for 5 minutes at 56°C apparently retards somewhat the clotting times, indicating possibly a destruction of the labile factor* or partial inactivation of the other factors present in the eluate. Here again, a similarity in the clotting times of the eluate with either platelets and $CaCl_2$ or heated thromboplastin was observed. The clotting of unheated and heated eluate with $CaCl_2$ alone is believed to indicate the presence of the platelet factor in the eluate.

As can be seen from the data of Table 2, the $BaSO_4$-eluate with fibrinogen gave clotting times shorter than those of the plasma containing similar concentrations of the coagulation factors. The difference in the observed clotting times is probably due to the presence of certain anticoagulant factors in blood (plasma) which might delay the clotting process in blood (plasma).

Recombination of the $BaSO_4$-eluate with the plasma from which the eluate was prepared resulted in clotting times identical with those of normal plasma which was diluted to the same degree (see Table 2). These findings indicate that there is apparently no loss in coagulation activity during the preparation of the eluate.

It would seem that the following coagulation factors are removed by $BaSO_4$: proconvertin, prothrombin, platelet factor and perhaps traces of the labile factor. There is general agreement that prothrombin and proconvertin can be removed from plasma by $BaSO_4$ (3, 4, 5).

*It is quite possible that small amounts of the labile factor may be adsorbed on $BaSO_4$, especially in cases where the plasma may contain a comparatively high concentration of this factor.

Campbell and Stefanini (9) recently reported that $BaSO_4$ removes the platelet factor from citrated plasma, while prothrombin and proconvertin are not adsorbed under the same experimental conditions. Attempts to remove the platelet factor from citrated rabbit plasma under the same experimental conditions or by Berkefeld filtration, as reported by Eagle (10), were only partially successful (see Table 3).

Apparently other factors, in addition to the platelet-factor, are at least partially removed by these procedures. Addition of varying concentrations of platelets to either Berkefeld filtered oxalated plasma or $BaSO_4$-treated citrated plasma definitely increased the rate of thrombin formation, which could be further accelerated by increasing the concentration of the labile factor. (see Table 3).

In passing it may be mentioned that it was observed that $BaSO_4$ also apparently adsorbs plasminogen. This factor, by becoming activated to plasmin, may account for the destruction of prothrombin frequently observed on attempts to isolate prothrombin from $BaSO_4$ eluates.

Table 4 shows that upon aging, plasma will show a reduction in the velocity of prothrombin conversion, caused by the deterioration of the labile plasma factor. This defect can be rectified by the addition of fresh oxalated plasma or fresh prothrombin-free ($BaSO_4$-treated) plasma. These data confirm similar observations of other investigators (11, 12, 13). Apparently, the factor which becomes inactivated on aging is not readily adsorbed on $BaSO_4$ which is in agreement with the results of other investigators (13, 14, 15). It appears from these data that human plasma has a comparatively low content of labile factor compared with rabbit plasma. These observations agree with the results obtained on aging of plasma from different species (see Table 7).

Serum, obtained from either fresh blood or recalcified oxalated plasma, then treated with $BaSO_4$, does not greatly shorten the clotting time of aged plasma. Similar observations have been reported by Alexander and his associates (14). Apparently the labile factor, which deteriorates on aging, is utilized during normal clotting.

Defibrination of $BaSO_4$-treated, oxalated plasma by the addition of 20 units of thrombin per ml plasma, followed by another treatment with $BaSO_4$, apparently does not greatly affect the labile factor activity since this "serum" still exerts an accelerating effect on thrombin formation when added to aged plasma (Table 4). Honorato (16) has reported

that it is possible to reduce the prothrombin time of stored plasma to about normal levels by the addition of fresh plasma which has been treated with $Al(OH)_3$ and then with thrombin. The observation that the "serum" described above exerts an accelerating effect similar to that of $BaSO_4$-treated plasma contradicts the assumption of Ware, Fahey and Seegers (17) that the labile plasma Ac-globulin* is inactive as such but becomes activated under the influence of thrombin. The above finding also does not seem to substantiate the assumption of Owren (3) that the labile factor (proaccelerin) is inactive and becomes activated under the influence of thrombin to accelerin, which is supposed to govern the velocity of prothrombin conversion.

In this connection it may be mentioned that it has been found in this laboratory that commercial thrombin preparations contain proconvertin. It may be possible that the presence of this factor accounts partly for the accelerating effects observed after treatment with thrombin. The accelerating effect with fresh sera observed by various investigators (3, 4, 5) was confirmed, but is probably due to the presence of convertin. This effect will be discussed in more detail in a later section of this report.

The data recorded in Table 5 illustrate that the addition of a $BaSO_4$-eluate from either human or rabbit plasma to aged plasma caused no apparent acceleration in the clotting times. In spite of the increased amounts of prothrombin and proconvertin present, there is no definite change in the rate of thrombin formation. The above observation supports the finding of Alexander and his associates (14) that addition of serum-prothrombin-conversion-accelerator** to aged plasma did not influence clotting time, and also substantiates the finding of Owen and Bollman (15) that addition of purified prothrombin to oxalated plasma had no influence on the rate of thrombin formation.

Comparison of the clotting activity of $BaSO_4$-eluates from normal and aged plasmas (Tables 1 and 6) indicated that there were no pronounced differences in the clotting activity between the $BaSO_4$-eluates from normal plasma and from aged plasma. Heating of the $BaSO_4$-eluate from aged plasma for 5 minutes at 56°C caused reduction in clotting ability (Table 6). Again it was demonstrated that the platelet factor gave clotting times similar to those obtained with heated thromboplastin.

*This factor is now assumed to be identical with the labile factor (21).
**This factor is now assumed by Alexander to be a combination of proconvertin and convertin (18).

The data presented in Table 7 indicate that the concentration of the labile factor varied in different species. The concentration of this factor was highest in beef plasma and lowest in human plasma. According to Quick and Stefanini (19), rabbit plasma contains fifty times, and dog plasma ten times, as much labile factor as human plasma. Murphy and Seegers (20) have reported that the relative concentration of plasma Ac-globulin is low in human, higher in dog and rabbit and highest in bovine plasma. Alexander and his associates (14) found that human prothrombin diluted in bovine, rabbit, or dog prothrombin-free plasma ($BaSO_4$-treated) was converted to thrombin more rapidly than when diluted in human prothrombin-free plasma.

Data in Table 8 show that addition of various concentrations of platelet factor to aged plasma resulted in reduced clotting times. At certain concentrations of the platelet factor one could obtain clotting times similar to the recalcification time of normal oxalated plasma. Simultaneous addition of labile factor greatly enhanced the rate of thrombin formation, giving actually shorter clotting times than those found on recalcification of normal plasma. Stefanini and Crosby (22) reported that the amount of labile factor utilized during coagulation is directly proportional to the degree of thrombin formation. Depending on the content of labile factor in plasma, it may be possible that a certain proportion is not utilized during thrombin formation and therefore may be present in serum.

From the data presented in Table 9, it appears that the platelet factor is somewhat more stable than the labile factor at elevated temperatures.

Jacox (23) has described the formation of a "prothrombin-converting" factor upon addition of thromboplastin to serum. According to Jacox, this factor causes conversion of prothrombin in the virtual absence of calcium but is unable to cause direct conversion of fibrinogen to fibrin. Following its liberation from serum by thromboplastin, the factor slowly decreases in activity but can be reactivated by adding more thromboplastin to the inactive serum-thromboplastin mixture.

These investigations have been extended in this laboratory by employing the platelet factor instead of thromboplastin and by studying the effect of platelets on a $BaSO_4$-eluate of serum.

As can be seen from the data of Table 10, the mixture of serum (4-1/2 hrs) and platelets caused rapid coagulation of normal oxalated plasma but not of $BaSO_4$-treated oxalated plasma. Tests of this reaction mixture against fibrinogen indicated the presence of a small

amount of thrombin which is not sufficient, however, to explain the effect of the reaction mixture on normal oxalated plasma. Addition of serum alone or a mixture of $BaSO_4$-treated serum and platelets to normal oxalated plasma did not produce any clot (Table 10). Therefore, the coagulation of oxalated normal plasma by the serum-platelet mixture cannot be explained as a separate action of either component, serum or platelets. From this evidence it can be postulated that addition of platelets to serum initiates the formation of a factor which is capable of converting prothrombin to thrombin. The mechanism of this reaction probably consists of an interaction of the platelet factor with proconvertin, present in serum, to yield convertin which has the ability to convert prothrombin to thrombin. Further evidence for this assumption will be presented in the following experiments.

It can also be seen from Table 10 that after a certain period of incubation the serum-platelet mixture no longer caused coagulation of normal oxalated plasma. Apparently the convertin formed was inhibited by a serum-antifactor (anticonvertin) since this decrease in activity was not observed on incubation of a serum $BaSO_4$-eluate with platelets and $CaCl_2$ (see Table 13).

As can be seen from Table 11, the rate of convertin formation was dependent upon the temperature of the incubation mixture and a certain incubation period was necessary before maximum convertin activity was attained. Apparently, the rate of interaction between convertin and anti-convertin was also related to the temperature of the reaction mixture.

The addition of fresh serum to normal oxalated plasma caused rapid coagulation, but had no effect on $BaSO_4$-treated plasma (Table 12). Small amounts of thrombin were nevertheless present since this serum would clot fibrinogen. However, the amount of thrombin present was not sufficient to produce the rapid clotting time observed on the addition of fresh serum to normal oxalated plasma. These findings confirm the observation of Bordet and Gengou in 1904 (24) that "fresh" serum coagulated whole oxalated plasma much more rapidly than it did oxalated plasma from which prothrombin was removed by adsorption with tricalcium phosphate*.

It is assumed that this coagulation effect of "fresh" serum on normal oxalated plasma was brought about by convertin which was formed during the clotting of blood by the action of the platelet factor

*It is now known that proconvertin is also removed by tricalcium phosphate.

on proconvertin, convertin then converting prothrombin to thrombin. The data of Table 12 also illustrate that the coagulation effect of "fresh" serum on normal oxalated plasma gradually disappeared, possibly due to the action of an inhibitor on convertin. Addition of platelets to the serum at this point restored the clotting ability of the serum on normal oxalated plasma, which gradually disappeared again on standing. Jacox and Bays (25) have reported similar observations employing tissue thromboplastin* in place of platelets (see also reference 23). All of these observations indicate a similarity in the action of tissue thromboplastin and the platelet factor, i.e., both react with proconvertin to yield convertin which in turn has the ability to convert prothrombin to thrombin.

Alexander and his associates (26) have reported that when freshly drawn blood is added immediately to thromboplastin, this serum often contains much more serum-prothrombin-conversion-accelerator (SPCA)** than the serum of blood allowed to clot spontaneously. Reference has already been made to the finding of Jacox (23) that addition of thromboplastin to serum will yield a factor which has the ability to convert prothrombin to thrombin. The increased SPCA activity, observed by Alexander and his associates, is therefore probably caused by an increased convertin formation.

Seegers and his associates (20, 27) reported that in dog and human serum the Ac-globulin activity definitely decreased from the time the blood was drawn, virtually disappearing within 30 minutes after coagulation. It appears likely that the serum Ac-globulin, described by Seegers and his associates, is identical with convertin and that the inactivation of this factor is the result of an interaction with an anti-factor (anticonvertin) present in serum. Seegers and his associates (27) have already pointed out the similarity in the stability of human serum Ac-globulin and that of factor VI*** by Owren (28) and suggested that these two factors may be identical.

The data in Table 13 indicate that a serum-$BaSO_4$-eluate, $CaCl_2$ and platelet mixture will yield results similar to those obtained with a serum-platelet combination. However, as already pointed out, no disappearance of the convertin activity was observed, indicating that the anticonvertin factor is not adsorbed on $BaSO_4$. Again, as with the serum-platelet mixture, one can observe that a certain incubation period is necessary before full convertin activity is attained.

*Studies in this laboratory with thromboplastin instead of platelets fully confirmed the findings of Jacox (23) and of Jacox and Bays (25).

**Alexander now assumes that SPCA activity is a combination of convertin and proconvertin (18).

***Factor VI is now assumed by Owren to be identical with convertin (3).

The amount of $CaCl_2$ employed in these tests is not sufficient to produce any recalcification effect. However, it is apparent from the data that Ca++ is necessary for the formation of convertin as well as for the conversion of prothrombin to thrombin by convertin. Lewis and Ferguson (29) also observed that convertin activity can apparently be demonstrated only in the presence of calcium.

The data of Table 14 show that when the active convertin preparation was added to plasmas containing decreased amounts of prothrombin, the clotting times were correspondingly prolonged. The amount of $CaCl_2$ employed was not sufficient to produce any recalcification effect. These findings show that the clotting effect of convertin is related to the prothrombin concentration and are further evidence that the function of convertin is to convert prothrombin to thrombin.

The concept that convertin acts as a converting factor of prothrombin to thrombin is further substantiated by observations of its effects on aged plasma. As can be seen in Table 15, addition of a mixture of convertin and $CaCl_2$, sufficient for recalcification, to aged plasma (with and without labile factor) produced clotting times comparable with those obtained with the corresponding normal plasma. The concentration of the platelet factor employed for the convertin formation is not sufficient to significantly influence the clotting times. Apparently, the labile factor does not influence to any significant extent the rate of conversion of prothrombin to thrombin by convertin.

If a mixture of convertin and $CaCl_2$, insufficient for recalcification, is tested against normal or aged (with and without addition of labile factor) rabbit plasma, clotting times are obtained which are longer than those obtained with the higher amount of $CaCl_2$. This is further evidence that the rate of prothrombin conversion by convertin is related to the presence and concentration of calcium ions. Tested against normal or aged (with and without labile factor) human plasma, no clot formation was observed in the aged plasma samples, while normal plasma gave a clotting time of 1'29". At present no explanation can be given for the difference in this reaction. It is evident that the labile factor employed is active since addition of this factor to aged rabbit plasma gave a recalcification time even shorter than that of normal plasma (see Table 15)

Preliminary experiments indicate that convertin is a rather labile substance. Thrombokinase, described by Milstone (30), and "blood thromboplastin", obtained by Biggs, Douglas and MacFarlane (31), were also found to be comparatively labile. These substances have in

common an action similar to that of convertin, and it is probable that these three factors are identical.

The data in Table 16 indicate that the labile factor accelerates the rate of convertin formation, but does not appear indispensable for this reaction. At the same time, in the presence of the labile factor, the "convertin" activity apparently becomes more stabilized since it is maintained longer. The mechanism of the latter reaction has still to be elucidated. The accelerating effect of the labile factor on the formation of convertin from proconvertin by the action of the platelet factor is further demonstrated by the data in Tables 3 and 8.

IV. DISCUSSION

On the basis of the studies reported here, and of certain findings reported in the literature (3, 4, 5, 32) and partly referred to above, the following scheme of "physiological" fibrin formation is suggested:

LABILE FACTOR
↓

1.	Proconvertin*	$\xrightarrow[Ca^{++}]{\text{Platelet Factor}}$	Convertin*
2.	Prothrombin	$\xrightarrow[Ca^{++}]{\text{Convertin}}$	Thrombin
3.	Fibrinogen	$\xrightarrow{\text{Thrombin}}$	Fibrin

These reactions are dependent upon each other in the order given, each reaction being a necessary forerunner of the next. When the last reaction has come into play, all three reactions will proceed simultaneously**. The clotting inhibiting factors such as antiplatelet factor, anticonvertin, antithrombin, heparin co-factor and perhaps others have been omitted from the above diagram for reason of simplicity. The labile factor is apparently utilized during clotting and is absent in serum. It seems that this factor accelerates the conversion of proconvertin to convertin by the platelet factor, but does not appear indispensable for this reaction. Calcium ions seem to play a role in the formation of convertin as well as of thrombin.

Inasmuch as tissue thromboplastin enters the blood clotting process only in cases of emergency such as injury, it may be advisable to

*Inasmuch as these factors were first discussed by Owren (3), his terminology has been applied.

**A three-step scheme has also been proposed by Milstone (4)

separate the normal physiological clotting mechanism from that occuring in cases of injury in which tissue thromboplastin comes into play. It has already been indicated that tissue thromboplastin, apparently, does not act directly on prothrombin but first interacts in the presence of calcium with proconvertin to produce the prothrombin converting factor, convertin.

The rate of normal fibrin formation in blood is then controlled by the proper physiological coordination of those agents which favor fibrin formation (as shown in the above scheme) and of those factors opposing fibrin formation (previously enumerated). Any relative or absolute deficiency or preponderance of certain factors may result in a definite abnormal shift of fibrin formation and may lead either to hemorrhagic- or thrombotic-like conditions.

There are apparently definite variations in the concentration of the various blood clotting factors, those favoring and those opposing fibrin formation, in different species. However, there is apparently no difference in the nature and function of the factors involved in blood clotting. Results obtained in one species cannot indiscriminately be transferred to another species.

The normal physiological process of fibrin formation may proceed at a comparatively slow rate. On the other hand, in cases of injury when tissue thromboplastin enters into the clotting mechanism the rate of formation of convertin and consequently that of thrombin and fibrin becomes accelerated. In addition, thrombin will then further accelerate its own production by increasing the rate of platelet disintegration (33, 34). There exists apparently a balance between thrombin formation and the rate of platelet disintegration. Thus, it appears that thrombin is the true "autocatalytic" accelerator of prothrombin conversion.

In addition to those factors favoring the process of fibrin formation there are agents which oppose this process. Physiological anticoagulants play an apparently important role in regulating the speed and completeness of fibrin formation. Finally, as pointed out in the introduction, the plasmin-antiplasmin system competes with the process of fibrin formation.

On the basis of the findings recorded here and of a re-evaluation of the various factors postulated to be involved in the process of thrombin formation, the following lists of synonyms are presented:

A. Synonyms for Labile Factor

Thrombogène - Nolf. P., Arch. Int. Physiol. 6: 115, 1908.

Prothrombin Accelerator - Fantl, P., and Nance, M. H. Nature 158: 708, 1946.

Labile Factor (Prothrombin A of 1943) - Quick, A. J., Am. J. Physiol. 151: 63, 1947.

Plasma Ac-globulin - Ware, A. G., Guest, M. M., and Seegers, W. H., J. Biol. Chem. 169: 231, 1947.

Plasmatic Co-Factor of Thromboplastin - Honorato, C. R., Am. J. Physiol. 150: 381, 1947.

Proaccelerin (Factor V) - Owren, P. A., and Aas, K., Scandinav. J. Clin. & Lab. Invest. 3: 201, 1951.

Plasma-Prothrombin-Conversion-Factor (PPCF) - Stefanini, M., Blood 6: 84, 1951.

B. Synonyms for Proconvertin

Plasmakinin - Laki, K., Schweiz, med. Wchnschr. 74: 13, 1944.

Thromboplastinogen - Quick, A. J., Am. J. Med. Sci. 214: 272, 1947.

Antihemophilic Factor (Plasma component which reacts with platelets) - Cohn, E. J., Experentia 3: 125, 1947.

Thrombocytolysin - Brinkhous, K. M., Proc. Soc. Exper. Biol. Med. 66: 117, 1947.

Prothrombin Conversion Factor (Accelerator) - Owen, C. A., and Bollman, J. L., Proc. Soc. Exper. Biol. Med. 67: 231, 1948.

Prothrombin Accelerator Factor - MacMillan, R. L., Science 108: 416, 1948.

Co-Thromboplastin - Mann, F. D., Am. J. Clin. Pathol. 19: 861, 1949.

Thromboplastin Precursor - Conley, C. L., Hartman, R. C., and Morse, W. I., J. Clin. Invest. 28: 340, 1949.

Precursor Serum Prothrombin Conversion Accelerator (SPCA) - Alexander, B., Goldstein, R., and Landwehr, G., J. Clin. Invest. 29: 881, 1950.

Stable Prothrombin Conversion Factor - Owen, Jr., C.A. Magath, T.B., and Bollman, J.L., Am. J. Physiol. 166: 1, 1951.

Thromboplastic Plasma Component (TPC) - Shinowara, G.Y., J. Lab. and Clin. Med. 38: 11, 1951.

Plasma Thromboplastic Factor - Ratnoff, O.D., and Conley, C.L., Bull. Johns Hopkins Hosp. 89: 245, 1951.

Proconvertin (Co-Factor V) - Owren, P.A., and Bjerkelund, C., Scandinav. J. Clin. and Lab. Invest. 1: 131, 1949.

Factor VII - Koller, F., and Loeliger, A., Helv. physiol. et pharmacol. acta 9: 27, 1951.

C. Synonyms for Platelet Factor

Cytozyme - Bordet, J., Ann. Inst. Pasteur 34: 561, 1920.

Thromboplastin Protein - Chargaff, E., and West, R., J. Biol. Chem. 162: 189, 1946.

Thromboplastinogenase - Quick, A.J., Am. J. Med. Sci. 214: 272, 1947.

Thromboplastic Cell Component (TCC) - Shinowara, G.Y., J. Lab. and Clin. Med. 38: 11, 1951.

Plasma Thromboplastin - Campbell, E.W., and Stefanini, M., Proc. Soc. Exper. Biol. Med. 83: 105, 1953.

D. Synonyms for Convertin

Serum Ac-Globulin - Murphy, R.C., Ware, A.G., and Seegers, W.H., Proc. Soc. Exper. Biol. Med. 69: 216, 1948.

Prothrombin Converting Factor - Jacox, R.F., J. Clin. Invest. 28: 492, 1949.

Serum Prothrombin Conversion Accelerator (SPCA) - Alexander, B., Goldstein, R., and Landwehr, G. J. Clin. Invest. 29: 881, 1950.

Factor VI (Convertin) - Owren, P.A., Scandinav. J. Clin. and Lab. Invest. 2: 241, 1950.

Thrombokinase - Milstone, J.H., Yale J. Biol. and Med. 25: 19, 1952.

Blood Thromboplastin - Biggs, R., Douglas, A.S., and MacFarlane, R.G., J. Physiol. 119: 89, 1953.

Several other proposed factors have not been well enough defined or identified and most of them are probably mixtures of certain factors listed. It seems advisable to explore the possibility of arriving at some agreement regarding the identity or nonidentity of the various factors reported to be involved in the physiological conversion of prothrombin to thrombin.

V. CONCLUSIONS

On the basis of the studies reported here, the following scheme of "physiological" fibrin formation is suggested:

LABILE FACTOR

1.	Proconvertin	$\xrightarrow[Ca^{++}]{\text{Platelet Factor}}$	Convertin
2.	Prothrombin	$\xrightarrow[Ca^{++}]{\text{Convertin}}$	Thrombin
3.	Fibrinogen	$\xrightarrow{\text{Thrombin}}$	Fibrin

The labile factor seems to exert an accelerating effect on the rate of conversion of proconvertin to convertin by the platelet factor, but does not appear indispensable for this reaction. Calcium ions seem to play a role in the formation of convertin as well as of thrombin.

A re-evaluation of the various factors which have been postulated to be involved in the process of thrombin formation has been presented.

VI. RECOMMENDATIONS

In order to have a reliable basis for a more complete understanding of the blood clotting mechanism, it is imperative to isolate the various factors in as chemically pure form as possible. Only when the physiological-chemical reaction brought about by the interaction of these factors can be studied in clearly defined systems, will it be possible to precisely formulate the clotting mechanism.

BIBLIOGRAPHY

1. Jensen, H., D. L. Chamovitz, E. Volkringer, E. Gray, J. E. Gosney, and W. Kocholaty. Antifibrinolytic activity in plasma after exposure to cold and after chymotrypsin administration. Blood 8: 324, 1953.

2. Allen, J. G., P. V. Moulder, D. M. Enerson, and D. Glotzer. The dynamics of coagulation. J. Lab. and Clin. Med. 34: 1579, 1949.

3. Owren, P. A. Prothrombin and accessory factors, clinical significance. Am. J. Med. 14: 201, 1953.

4. Milstone, J. H. On the evolution of blood clotting theory. Med. 31: 411, 1952.

5. Stefanini, M. Mechanisms of blood coagulation in normal and pathologic conditions. Am. J. Med. 14: 64, 1953.

6. Wolf, P. A modification for routine laboratory use of Stefanini's method of estimating factor V activity in human oxalated plasma. J. Clin. Path. 6: 1, 1953.

7. Owren, P. A. The fifth coagulation factor ("Factor V"). Preparation and properties. Biochem. J. 43: 136, 1948.

8. Ware, A. G., M. M. Guest, and W. H. Seegers. Fibrinogen: With special reference to its preparation and certain properties of the product. Arch. Biochem. 13: 231, 1947.

9. Campbell, E. W., and M. Stefanini. Isolation and purification of plasma thromboplastin: Its physico-chemical and biologic properties. Proc. Soc. Exper. Biol. Med. 83: 105, 1953.

10. Eagle, H. Studies on blood coagulation: Role of prothrombin and of platelets in formation of thrombin. J. Gen. Physiol. 18: 531, 1934/35.

11. Quick, A. J., and M. Stefanini. The chemical state of the calcium reacting in the coagulation of blood. J. Gen. Physiol. 32: 191, 1948.

12. Alexander, B., and A. deVries. Human prothrombin: Quantitative studies on the plasma labile factor and the restorative effects of normal, hypofibrinogenemic, and hemophilic plasma on the prothrombin of stored plasma. J. Clin. Invest. 28: 24, 1949.

13. Loomis, E. C., and W. H. Seegers. Is prothrombin a unitary principle or a complex? Am. J. Physiol. 148: 563, 1947.

14. Alexander, B., R. Goldstein, and G. Landwehr. The prothrombin conversion accelerator of serum (SPCA): Its partial purification and its properties compared with serum Ac-globulin. J. Clin. Invest. 29: 881, 1950.

15. Owen, Jr., C. A., and J. L. Bollman. Analysis of the one-stage test for prothrombin time. J. Applied Physiol. 5: 515, 1953.

16. Honorato, R. The plasmatic co-factor of thromboplastin: Its adsorption, with prothrombin and fibrinogen, by alumina and tricalcium phosphate gels. Am. J. Physiol. 150: 381, 1947.

17. Ware, A. G., J. L. Fahey, and W. H. Seegers. Platelet extracts, fibrin formation and interaction of purified prothrombin and thromboplastin. Am. J. Physiol. 154: 140, 1948.

18. Alexander, B. Blood clotting and allied problems. Flynn, J. E., Editor. Trans. Fifth Conf., New York, Josiah Macy, Jr., Foundation, 1952, pp. 111-129.

19. Quick, A. J., and M. Stefanini. The concentration of the labile factor of the prothrombin complex in human, dog, and rabbit blood; its significance in the determination of prothrombin activity. J. Lab. and Clin. Med. 33: 819, 1948.

20. Murphy, R. C., and W. H. Seegers. Concentration of prothrombin and Ac-glogulin in various species. Am. J. Physiol. 154: 134, 1948.

21. Blood clotting and allied problems. Flynn, J. E., Editor. Trans. Fifth Conf., New York, Josiah Macy, Jr., Foundation, 1952, pp. 329-333.

22. Stefanini, M., and W. H. Crosby. Utilization of the labile factor during normal and abnormal coagulation of blood. Proc. Soc. Exper. Biol. Med. 74: 370, 1950.

23. Jacox, R. F. Studies on the activation of a serum "prothrombin-converting factor". J. Clin. Invest. 28: 492, 1949.

24. Bordet, J., and O. Gengou. Recheres sur la coagulation du sang; sur le pouvoir coagulant du serum. Ann. Inst. Pasteur 18: 98, 1904.

25. Jacox, R. F., and R. P. Bays. Studies of the "thrombin" effect of fresh serum. Proc. Soc. Exper. Biol. Med. 70: 587, 1949.

26. DeVries, A., B. Alexander, and R. Goldstein. A factor in serum which accelerates the conversion of prothrombin to thrombin: I. Its determination and some physiologic and biochemical properties. Blood 4: 247, 1949.

27. Murphy, R. C., A. G. Ware, and W. H. Seegers. Stability of serum Ac-globulin. Proc. Soc. Exper. Biol. Med. 69: 216, 1948.

28. Owren, P. A. The coagulation of blood; investigations on a new clotting factor. Acta med. scandinav. Supp. 194, 1947, pp. 1-327.

29. Lewis, J. H., and J. H. Ferguson. Partial purification of human prothrombin and proconvertin: Their characteristics and interaction. J. Clin. Invest. 32: 915, 1953.

30. Milstone, J. H. Protein thrombokinase and lipoid thromboplastin as distinct factors with complementary functions. Yale J. Biol. and Med. 25: 19, 1952.

31. Biggs, R., A. S. Douglas, and R. G. MacFarlane. The formation of thromboplastin in human blood. J. Physiol. 119: 89, 1953.

32. Seegers, W. H. Blood coagulation. The enzymes, chemistry and mechanism of action, edited by James B. Sumner and Karl Myrback, Vol. I, Part 2, Academic Press, Inc., Publishers, New York, 1951, pp. 1106-1155.

33. Stefanini, M. Autocatalytic formation of thrombin in blood coagulation. Acta. med. scandinav. 140: 290, 1951.

34. DeRobertis, E., P. Passeyro, and M. Reissig. Electron microscopic studies of the action of thrombin on blood platelets. Blood 8: 587, 1953.

TABLE 1

EFFECT OF HEAT (56°C. 5 Min) ON $BaSO_4$-ELUATE FROM NORMAL PLASMA

TEST MIXTURE*	CLOT FORMATION TIMES*** Normal Plasma Eluate Unheated 1-Stage	Normal Plasma Eluate Unheated 2-Stage	Normal Plasma Eluate Heated 1-Stage	Normal Plasma Eluate Heated 2-Stage
RABBIT:				
Eluate + Fibrinogen**	NC30'****	NC30'	NC30'	NC30'
" + $CaCl_2$ + Fibrinogen	2'57''	2'31''	4'36''	3'40''
" + $CaCl_2$ + Platelets + Fibrinogen	1'12''	13''	2'6''	20''
" + Unheated Thromboplastin + Fibrinogen	36''	10''	1'5''	18''
" + Heated Thromboplastin + Fibrinogen	2'50''	17''	4'20''	30''
HUMAN:				
Eluate + Fibrinogen	NC30'	NC30'	NC30'	NC30'
" + $CaCl_2$ + Fibrinogen	4'17''	3'3''	8'25''	6'26''
" + $CaCl_2$ + Platelets + Fibrinogen	3'0''	45''	4'57''	1'21''
" + Unheated Thromboplastin + Fibrinogen	55''	8''	1'23''	20''
" + Heated Thromboplastin + Fibrinogen	3'15''	1'0''	5'40''	1'50''

* Final volume of test mixture 1.1 ml.
0.1 ml $BaSO_4$-Eluate (plasma concentration)
0.1 ml 0.025 M $CaCl_2$
0.1 ml Platelets (6 x blood concentration)
0.2 ml Thromboplastin
0.9% NaCl (amount necessary to give final volume of 1.1 ml)

** 0.7 ml Fibrinogen

*** Clot formation times in all tables observed at 37°C unless otherwise specified

**** NC = No clot formed at time indicated.

TABLE 2

TESTS ON $BaSO_4$-ELUATES FROM NORMAL PLASMA

TEST MIXTURE*	CLOT FORMATION TIMES** Rabbit	Human
A. $BaSO_4$-Eluate (volume equivalent to 1.0 ml plasma) + 1.0 ml 0.4% fibrinogen		
A + saline + $CaCl_2$	1'35''	2'49''
A + platelets + $CaCl_2$	1'2''	1'27''
A + heated thromboplastin	36''	2'40''
B. 0.9% NaCl (volume equivalent to volume of eluate used above) + 1.0 ml normal plasma.		
B + saline + $CaCl_2$	3'16''	5'13''
B + platelets + $CaCl_2$	1'50''	3'3''
B + heated thromboplastin	42''	1'30''
C. 1.0 ml $BaSO_4$-Eluate (plasma concentration) + 1.0 ml $BaSO_4$ treated plasma		
C + saline $CaCl_2$	5'12''	5'18''
C + platelets + $CaCl_2$	1'15''	1'22''
C + heated thromboplastin	52''	1'28''
D. 1.0 ml 0.9% NaCl + 1.0 ml normal plasma.		
D + saline + $CaCl_2$	4'53''	5'55''
D + platelets + $CaCl_2$	1'50''	2'50''
D + heated thromboplastin	47''	1'48''

* Test Mixture:
A,B,C, or D 0.2 ml in tests with $CaCl_2$ and $CaCl_2$ + platelets.
A,B,C, or D 0.1 ml in tests with thromboplastin
0.025 M $CaCl_2$ 0.2 ml
Platelets (6 x concentration in blood) 0.1 ml
Heated thromboplastin 0.2 ml
0.9% saline 0.1 ml

** Clot formation times recorded in the various tables were obtained by the one-stage technique unless otherwise specified

TABLE 3

INFLUENCE OF THE CONCENTRATION OF PLATELETS AND/OR LABILE FACTOR ON THE CLOTTING TIME OF VARIOUS RABBIT PLASMAS

TEST MIXTURE*	Normal Oxalated Plasma	Berkefeld Filtered Oxalated Plasma	Normal Citrated Plasma	$BaSO_4$-Treated Citrated Plasma
Saline + $CaCl_2$	3'25''	18'20''	3'0''	12'32''
Platelets (10 x conc) + $CaCl_2$	34''	1'18''	40''	1'54''
Platelets (5 x conc) + $CaCl_2$	43''	1'20''	42''	2'18''
Platelets (3 x conc) + $CaCl_2$	55''	1'42''	45''	2'40''
Platelets (1 x conc) + $CaCl_2$	1'0''	2'0''	46''	2'48''
Labile Factor (2 mg/0.1 ml) + $CaCl_2$	1'39''	2'37''	1'40''	5'9''
Labile Factor (1 mg/0.1 ml) + $CaCl_2$	2'26''	3'47''	1'51''	6'19''
Labile Factor (0.5 mg/0.1 ml) + $CaCl_2$	3'1''	5'30''	2'9''	8'7''
Labile Factor (0.25 mg/0.1 ml) + $CaCl_2$	3'30''	7'45''	2'39''	10'11''
Platelets (10 x conc) + Labile Factor (2 mg/ 0.1 ml) + $CaCl_2$	24''	35''	27''	35''
Platelets (1 x conc) + Labile Factor (0.25 mg/0.1 ml) + $CaCl_2$	54''	1'16''	55''	1'30''
Thromboplastin**	12''	12''	11''	19''
Heated Thromboplastin	50''	58''	25''	36''

* Final Volume - 0.6 ml
Plasma - 0.2 ml
$CaCl_2$ (0.025 M) - 0.2 ml
Labile Factor - 0.1 ml
Platelets - 0.1 ml
Saline - amount necessary to bring final volume to 0.6 ml.

** Thromboplastin tests - 0.1 ml plasma + 0.2 ml thromboplastin.

TABLE 4

INFLUENCE OF ADDITION OF DIFFERENT PLASMAS AND SERA ON CLOTTING TIMES OF AGED PLASMA

TEST MIXTURE*	CLOT FORMATION TIMES: Saline + $CaCl_2$		Platelets + $CaCl_2$		Heated Thromboplastin	
Aged Human Plasma	NC30'		4'49''		3'22''	
	Human	Rabbit	Human	Rabbit	Human	Rabbit
Normal Plasma	4'5''	2'18''	1'54''	45'''	1'26''	41''
Aged Plasma + Normal Plasma**	11'4''	5'5''	2'34''	1'52''	2'12''	1'8''
" " + $BaSO_4$-Treated Plasma	20'24''	13'46''	3'6''	2'23''	2'1''	1'18''
" " + $BaSO_4$-Treated Serum I***	NC30'	NC25'	4'55''	4'17''	3'24''	2'43''
" " + $BaSO_4$-Treated Serum II	NC30'		4'12''		3'52''	
" " + $BaSO_4$-Treated Serum III	18'4''	9'49''	3'0''	1'52''	3'11''	2'21''

* For test mixture see Table 2

** Ratio of mixtures 4:1

*** Serum I Fresh blood clotted
Serum II Recalcification of oxalated plasma
Serum III Defibrination of $BaSO_4$-treated oxalated plasma by addition of thrombin (20 units/ml plasma)

TABLE 5

INFLUENCE OF $BaSO_4$-ELUATE FROM NORMAL PLASMA ON CLOTTING BEHAVIOR OF AGED PLASMA

TEST MIXTURE*	CLOT FORMATION TIMES		
	Saline + $CaCl_2$	Platelets + $CaCl_2$	Heated Thromboplastin
Aged Rabbit Plasma	6'45''	1'38''	1'35''
Normal Rabbit Plasma	1'37''	42''	32''
Aged Rabbit Plasma + Normal Rabbit Plasma**	4'7''	50''	1'15''
Aged Rabbit Plasma + $BaSO_4$ Eluate-Rabbit Plasma	3'53''	1'4''	1'7''
Aged Rabbit Plasma + $BaSO_4$ Eluate-Human Plasma	6'38''	1'5''	1'20''
Aged Human Plasma	NC30'	6'18''	1'56''
Normal Human Plasma	3'40''	1'6''	41''
Aged Human Plasma + Normal Human Plasma	11'0''	2'17''	1'10''
Aged Human Plasma + $BaSO_4$ Eluate-Rabbit Plasma	25'20''	4'45''	1'12''
Aged Human Plasma + $BaSO_4$ Eluate-Human Plasma	NC30'	5'37''	1'53''

TEST MIXTURE***	RABBIT		HUMAN	
	1-Stage	2-Stage	1-Stage	2 Stage
$BaSO_4$ Eluate + Fibrinogen	21'50''	16'0''	NC30'	NC30'
" " + $CaCl_2$ + Fibrinogen	6'35''	4'2''	6'55''	6'25''
" " + $CaCl_2$ + Platelets + Fibrinogen	1'25''	15''	4'30''	1'7''
" " + Unheated Thromboplastin + Fibrinogen	51''	13''	51''	≤15''
" " + Heated Thromboplastin + Fibrinogen	2'30''	20''	2'31''	40''

* For test mixture see Table 2.

** Ratio of mixtures 4:1.

*** For test mixture see Table 1.

TABLE 6

EFFECT OF HEAT (58°C. 5 Min) ON $BaSO_4$-ELUATE FROM AGED PLASMA

TEST MIXTURE*	CLOT FORMATION TIMES: Aged Plasma Eluate Unheated 1-Stage	Aged Plasma Eluate Unheated 2-Stage	Aged Plasma Eluate Heated 1-Stage	Aged Plasma Eluate Heated 2-Stage
RABBIT:				
Eluate + Fibrinogen	NC30'	NC30'	NC30'	NC30'
" + $CaCl_2$ + Fibrinogen	4'32"	3'40"	7'32"	5'7"
" + $CaCl_2$ + Platelets + Fibrinogen	2'11"	18"	3'9"	1'57"
" + Unheated Thromboplastin + Fibrinogen	50"	12"	1'16"	21"
" + Heated Thromboplastin + Fibrinogen	3'18"	< 15"	4'15"	24"
HUMAN:				
Eluate + Fibrinogen	NC30'	NC30'	NC30'	NC30'
" + $CaCl_2$ + Fibrinogen	3'35"	2'31"	NC30'	6'2"
" + $CaCl_2$ + Platelets + Fibrinogen	1'45"	10"	4'1"	31"
" + Unheated Thromboplastin + Fibrinogen	57"	9"	2'6"	21"
" + Heated Thromboplastin + Fibrinogen	3'15"	1'17"	4'57"	1'57"

* For test mixture see Table 1

TABLE 7

EFFECT OF AGING OF PLASMA FROM DIFFERENT SPECIES

TYPE OF PLASMA*	CLOT FORMATION TIMES: Saline + $CaCl_2$	Platelets + $CaCl_2$	Heated Thromboplastin	Normal Thromboplastin
Normal Human Plasma	3'59"	1'14"	1'38"	18"
Aged Human Plasma	NC30'	3'19"	6'15"	44"
Normal Rabbit Plasma	1'54"	50"	44"	10"
Aged Rabbit Plasma	5'31"	1'23"	1'11"	14"
Normal Beef Plasma	1'30"	1'0"	49"	18"
Aged Beef Plasma	3'15"	2'31"	1'38"	19"
Normal Rat Plasma	2'26"	59"	1'25"	16"
Aged Rat Plasma	5'35"	2'7"	2'14"	23"
Normal Dog Plasma	1'57"	47"	40"	11"
Aged Dog Plasma	8'50"	2'24"	1'35"	22"

*Test mixture:		
	Final volume	0.6 ml
	Plasma	0.2 ml
	$CaCl_2$ (0.025 M)	0.2 ml
	Thromboplastin	0.2 ml
	Platelets (6 x blood conc)	0.1 ml
	0.9% Saline	Volume necessary to bring final volume to 0.6 ml.

TABLE 8

INFLUENCE OF VARIOUS PLATELET CONCENTRATIONS ON CLOTTING TIME OF AGED PLASMA CONTAINING A GIVEN AMOUNT OF LABILE FACTOR

TEST MIXTURE*	Saline $+CaCl_2$	Platelet Conc**	Platelets $+ CaCl_2$	Platelets + Labile Factor $+ CaCl_2$
Normal Human Plasma	4'10''			
Normal Human Plasma + Platelets		10x	1'35''	
Aged Human Plasma	NC30'			
Aged Human Plasma + Platelets		10x	3'50''	1'43''
		5x	3'55''	1'50''
		3x	4'10''	1'52''
		2x	4'37''	2'2''
		1x	4'50''	2'22''
		1/2x	6'43''	2'45''
		1/4x	8'15''	3'11''
Aged Human Plasma + Labile Factor	6'5''			

* For test mixture see Table 3
Labile factor 1 mg/0.1 ml

** Platelet concentration - multiple of blood concentration.

TABLE 9

EFFECT OF HEAT (56°C. 5 Min) ON PLATELET FACTOR AND LABILE FACTOR*

	Saline $+CaCl_2$	Thromboplastin	Heated Thromboplastin
Aged Plasma (Human)	15'15''	38''	3'12''
		Unheated Platelets $++CaCl_2$	Heated Platelets $+CaCl_2$
Aged Plasma + Platelets (10 x conc)		2'18''	3'1''
+ Platelets (5 x conc)		2'50''	6'17''
+ Platelets (3 x conc)		4'48''	8'33''
+ Platelets (1 x conc)		10'4''	11'57''
		Unheated Labile Factor $+ CaCl_2$	Heated Labile Factor $+ CaCl_2$
Aged Plasma + Labile Factor (2 mg/0.1 ml)		4'40''	8'55''
+ Labile Factor (1 mg/0.1 ml)		4'55''	10'10''
+ Labile Factor (0.5 mg/0.1 ml)		7'51''	13'0''
+ Labile Factor (0.25 mg/0.1 ml)		10'20''	14'45''

* Test mixture:

Final volume	0.6 ml
Plasma	0.2 ml
$CaCl_2$ (0.025 M)	0.2 ml
Thromboplastin	0.2 ml
Platelets	0.1 ml
Labile Factor	0.1 ml
0.9% Saline	volume necessary to bring final volume to 0.6 ml

TABLE 10

EFFECT OF SERUM-PLATELET MIXTURE ON OXALATED NORMAL PLASMA, $BaSO_4$-TREATED PLASMA AND FIBRINOGEN

INCUBATION MIXTURE*	CLOT FORMATION TIMES			
	Minutes Incubation	Oxalated Rabbit Plasma	$BaSO_4$-Treated Rabbit Plasma	Fibrinogen
Serum	0	NC20'	--	NC20'
Serum + Platelets**	1'	1'26''	NC30'	3'20''
	5'	1'7''	NC30'	6'7''
	10'	1'22''	NC30'	15'10''
	15'	2'0''	NC30'	23'10''
	20'	3'21''	NC30'	28'45''
	25'	22'0''	NC30'	NC30'
	30'	NC30'	NC30'	NC30'
	35'	NC30'	NC30'	NC30'
$BaSO_4$Treated Serum + Platelets**	1'-20'	NC20'	NC20'	NC20'
0.9% NaCl + $CaCl_2$ + Platelets***	1'-20'	NC20'	NC20'	NC20'

CONTROLS:	CFT
0.2 ml oxalated rabbit plasma + 0.2 ml 0.025 M $CaCl_2$	1'52''
0 2 ml $BaSO_4$-treated rabbit plasma + 0.2 ml 0.025 M $CaCl_2$	NC30'
0.2 ml $BaSO_4$-treated rabbit plasma + 1 unit thrombin	22''

* All mixtures incubated at 37°C.
Test Mixture: 0.1 ml incubation mixture + 0.2 ml plasma or fibrinogen.

** Serum (4-1/2 hrs) and platelets (10 x blood concentration) combined in ratio 5:1.

*** 0.9% NaCl 1.0 ml
0.125 M $CaCl_2$ 0.1 ml
Platelets 10 x blood concentration 0.2 ml

TABLE 11

INCUBATION OF RABBIT SERUM WITH PLATELETS AT 21°C AND 37°C

	Oxalated Rabbit Plasma				$BaSO_4$-Treated Rabbit Plasma			
	21°C		37°C		21°C		37°C	
Incubation Mixture*	Minutes Incubation	CFT**	Minutes Incubation	CFT	Minutes Incubation	CFT	Minutes Incubation	CFT
Serum + Platelets***	1'	4'4''	1'	1'47''	8'	NC30'	2'	NC30'
	5'	2'50''	4'	1'25''			8'	NC30'
	8'	2'18''	6'	1'10''			35'	NC30'
	11'	2'23''	9'	1'15''				
	15'	1'43''	12'	1'4''				
	17'	1'21''	16'	1'25''				
	20'	1'10''	20'	2'25''				
	24'	58''	24'	1'40''				
	28'	1'14''	28'	3'25''				
	32'	1'8''	32'	3'0''				
	36'	55''	37'	3'47''				
			40'	6'0''				
			47'	22'55''				
			60'	NC30'				

* Test Mixture: 0.2 ml incubation mixture + 0.2 ml plasma.

** CFT = Clot formation times.

*** Serum (4-1/2 hrs) and platelets (10 x blood concentration) combined in ratio 5:1.

TABLE 12

COAGULATION EFFECT OF FRESH SERUM AND SERUM-PLATELET MIXTURE ON OXALATED PLASMA AND FIBRINOGEN

	Clot Formation Time		
Test Mixture*	Minutes After Puncture	Oxalated Rabbit Plasma	Fibrinogen**
Fresh Serum (room temperature during testing)	45'	2'50''	7'35''
	50'	3'20''	10'26''
	55'	3'42''	9'9''
	60'	3'47''	9'53''
	65'	3'57''	10'20''
	70'	5'5''	10'5''
	75'	5'5''	13'15''
	80'	5'15''	11'33''
	90'	5'35''	12'30''
	110'	7'15''	12'37''
	130'	11'27''	10'15''
Serum + Platelets*** (incubated at 37°C during testing)	135'	53''	5'46''
	138'	48''	9'32''
	140'	49''	13'5''
	145'	1'0''	14'19''
	150'	1'15''	14'30''
	155'	1'31''	18'20''
	165'	2'8''	18'10''
	175'	3'18''	20'0''
	185'	13'12''	---

* 0.2 ml serum or incubation mixture + 0.2 ml plasma or fibrinogen.

** Fibrin strands only, no solid clots.

*** Serum and platelets (10 x blood concentration) combined in ratio 5:1.

TABLE 13

FORMATION OF A PROTHROMBIN CONVERTING FACTOR (CONVERTIN) ON INCUBATION OF SERUM-$BaSO_4$-ELUATE WITH PLATELETS AND THE IMPORTANCE OF CALCIUM IONS IN THE FORMATION AND EFFECT OF CONVERTIN

	INCUBATION MIXTURE*	Minutes Incubation	CLOT FORMATION TIMES: Oxalated Rabbit Plasma	$BaSO_4$-Treated Rabbit Plasma	Fibrinogen
I.	$BaSO_4$-Eluate-Serum + $CaCL_2$ + Saline	1'30''	NC30'	NC30'	17'-21'
II.	$BaSO_4$-Eluate-Serum + Platelets + Saline	1'-20''	NC20'	NC20'	NC20'
III.	$BaSO_4$-Eluate-Serum + $CaCl_2$ + Platelets**	1'	NC30'	NC30'	3'0''
		5'	50''	NC30'	1'25''
		10'	16''	7'2''	1'15''
		15'	14''	7'10''	59''
		20'	13''	6'20''	53''
		30'	11''	NC30'	44''
		40'	14''	NC30'	54''
		50'	15''	NC30'	56''
		60'	20''	NC30'	54''
Mixture III filtered through resin*** after 20 Min. incubation to remove Ca^{++}					
	2 ml filtrate + 0.1 ml 0.9% NaCl		NC30'	NC30'	7'43''
	2 ml filtrate + 0.1 ml 0.125 M $CaCl_2$ (0.066 mg $CaCl_2$/0.1 ml)		24''	NC30'	3'43''
	2 ml 0.9% NaCl + 0.1 ml 0.125 M $CaCl_2$		NC30'	NC30'	NC30'
	2 ml filtrate (heated at 56°C for 5 Min) + 0.1 ml 0.125 M $CaCl_2$		1'28''	--	14'39''
	2 ml filtrate (heated at 37°C for 5 Min) + 0.1 ml 0.125 M $CaCl_2$		31''	--	3'51''

* Test Mixture : 0.1 ml incubation mixture + 0.2 ml plasma or fibrinogen.

** Serum $BaSO_4$ - Eluate (2 x serum concentration) - 10 ml
0.125 M $CaCl_2$ - 1 ml
Platelets (20 x blood concentration) - 0.5 ml

*** Rohm and Haas IR-105 (Na)

TABLE 14

EFFECT OF CONVERTIN ON PLASMA CONTAINING DIFFERENT CONCENTRATIONS OF PROTHROMBIN

TEST MIXTURE*	CFT Convertin** + $CaCl_2$
Fibrinogen	5'20"
Normal Rabbit Plasma	36"
Normal Rabbit Plasma + $BaSO_4$-Treated Rabbit Plasma 3:1	49"
Normal Rabbit Plasma + $BaSO_4$-Treated Rabbit Plasma 1:1	1'7"
Normal Rabbit Plasma + $BaSO_4$-Treated Rabbit Plasma 1:3	1'30"

* 0.2 ml plasma or plasma mixture + 0.1 ml convertin-$CaCl_2$ mixture (2 ml convertin + 0.1 ml 0.125 M $CaCl_2$)

** Prepared by incubating serum $BaSO_4$-eluate with $CaCl_2$ and platelets and then filtering through resin IR-105 (Na) to remove Ca^{++}.

TABLE 15

EFFECT OF CONVERTIN ON AGED PLASMA

TEST MIXTURE*	CLOT FORMATION TIMES						
	Normal Rabbit Plasma	Aged Rabbit Plasma	Aged Rabbit Plasma + Labile Factor**	Normal Human Plasma	Aged Human Plasma	Aged Human Plasma + Labile Factor**	Fibrinogen
2 ml Convertin + 0.1 ml 0.9% NaCl	NC20'	NC20"	NC20'	NC20'	NC20'	NC20'	5'42"
2 ml Convertin + 0.1 ml 0.125 M $CaCl_2$***	41"	1'7"	58"	1'29"	NC20'	NC20'	3'1"
2 ml 0.9% NaCl + 0.1 ml 0.125 M $CaCl_2$	NC20'	NC20'	NC20'	NC20'	NC20'	NC20'	NC20'
2 ml Convertin + 0.1 ml 1.0 M $CaCl_2$****	29"	31"	26"	42"	1'33"	1'11"	3'25"
2 ml 0.9% NaCl + 0.1 ml 1.0 M $CaCl_2$	2'56"	4'25"	2'16"	5'20"	NC15'	8'23"	NC20'

* Test mixture: 0.1 ml mixture + 0.2 ml plasma or fibrinogen.

** Labile factor dissolved in aged plasma 1 mg/0.1 ml.

*** 0.066 mg $CaCl_2$/0.1 ml mixture.

**** 0.53 mg $CaCl_2$/0.1 ml mixture (recalcification concentration).

TABLE 16

EFFECT OF LABILE FACTOR ON FORMATION OF CONVERTIN IN SERUM (4-1/2 hrs) AT 26°C AND AT 37°C

INCUBATION MIXTURE*	Minutes Incubation	CLOT FORMATION TIMES: Oxalated Rabbit Plasma 26°C	Oxalated Rabbit Plasma 37°C	Fibrinogen 26°C	Fibrinogen 37°C
Serum	0'	NC20'	NC20'	NC20'	NC20'
Serum + Platelets	1'	2'30''	1'3''	10'50''	11'38''
	3'	1'4''	52''	19'20''	20'10''
	5'	46''	55''	20'15''	24'20''
	8'	43''	1'7''	NC20'	NC20'
	10'	43''	1'20''	NC20'	NC20'
	15'	45''	2'24''	NC20'	NC20'
	20'	50''	2'40''	NC20'	NC20'
	25'	1'5''	NC20''	NC20'	NC20'
	30'	1'9''	NC20'	NC20'	NC20'
	35'	1'15''	NC20'	NC20'	NC20'
	40'	1'40''	NC20'	NC20'	NC20'
	50'	1'36''	--	NC20'	--
	60'	2'5''	--	NC20'	--
Serum + Labile Factor**	0'	NC20'	NC20'	NC20'	NC20'
Serum-Labile Factor Mixture + Platelets	1'	1'5''	35''	10'50''	5'35''
	3'	39''	27''	14'59''	12'45''
	5'	31''	25''	14'25''	NC20'
	8'	28''	25''	15'0''	NC20'
	10'	29''	32''	NC20'	NC20'
	15'	33''	39''	NC20'	NC20'
	20'	36''	48''	NC20'	NC20'
	25'	42''	54''	NC20'	NC20'
	30'	45''	1'5''	NC20'	NC20'
	35'	51''	1'20''	NC16'	NC20'
	40'	56''	2'4''	NC11'	NC20'
	50'	53''	NC20'	--	NC20'
	60'	--	NC18'	--	NC18'
$BaSO_4$-Treated Serum-Labile Factor Mixture** + Platelets	1'-20'	NC20'	NC20'	NC20'	NC20'

* For test mixture and serum + platelets mixture see Table 10.

** Labile factor dissolved in serum 1 mg/0.1 ml.